

In Vitro Activities of Terbinafine in Combination with Fluconazole, Itraconazole, Voriconazole, and Posaconazole against Clinical Isolates of *Candida glabrata* with Decreased Susceptibility to Azoles

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A checkerboard microdilution method, performed according to the recommendations of the National Committee for Clinical Laboratory Standards, was used to study the in vitro interaction of terbinafine (TRB) with fluconazole (FLU), itraconazole (ITRA), voriconazole (VRC), and posaconazole (PSZ) in 24 isolates of *Candida glabrata* with decreased susceptibility to azoles isolated from the oral cavities of human immunodeficiency virus patients. Synergy, defined as a fractional inhibitory concentration index of ≤ 0.5 , was observed in 17% of TRB-FLU interactions, 21% of TRB-ITRA interactions, 33% of TRB-VRC interactions, and 12% of TRB-PSZ interactions. Where synergy was not achieved, there was still a decrease in the MIC of one or both drugs when used in combination. Antagonism was not observed in any drug combination. Clinical studies are warranted to elucidate the potential utility of these combination therapies.

Oropharyngeal candidiasis (OPC) caused by *Candida albicans* continues to be a common opportunistic infection in human immunodeficiency virus (HIV)-infected patients and is treated mainly with azole antifungal agents, particularly with fluconazole (FLU). The need for prolonged and repeated therapy has led to the emergence of fluconazole-resistant isolates of *C. albicans*, as well as the appearance of other, more resistant species, such as *Candida glabrata*, *Candida tropicalis*, and *Candida krusei* (3, 7). *C. glabrata* is a yeast with low intrinsic susceptibility to azole derivatives and is able to acquire resistance during treatment with these types of antifungal drugs (8, 11). An attractive therapeutic option in these circumstances might be a combination of antimicrobial agents with proven synergistic activity. Voriconazole (VRC) is an investigational triazole antifungal agent similar in structure and spectrum of action to FLU and itraconazole (ITRA). It has been shown to be more active than fluconazole and two- to eightfold more active than itraconazole against *Candida* species, including *C. krusei* and *C. glabrata* (13). Posaconazole (PSZ) is a new hydroxylated analogue of ITRA that has shown to have similar activity as its parental drug against *C. glabrata* (10). The allylamine terbinafine (TRB) has shown to have significant in vitro activity against *Candida parapsilosis*, although limited or no in vitro activity against *C. albicans*, *C. glabrata*, *C. krusei*, and *C. tropicalis* has been published (4, 12). Combination therapy is a promising novel approach in the therapy of candidiasis caused by strains resistant to conventional antifungal agents. Data on the effectiveness of combining TRB with the azoles are limited. Fothergill et al., in an in vitro pilot study, found a synergistic interaction between TRB and azoles (FLU and

ITRA) against *C. glabrata* isolates (A. W. Fothergill, I. Leitner, J. G. Meingasser, N. S. Ryder, and M. G. Rinaldi, Abstr. 36th Intersci. Conf. Antimicrob. Agents Chemother., abstr. E53, p. 91, 1996). It has been previously published that combinations of TRB with FLU, ITRA, and VRC against *C. albicans* isolates with reduced susceptibility to azoles have additive to synergistic effects against certain isolates and that no antagonistic effects were observed (1, 2, 14).

The aim of the present study was to investigate the in vitro interaction of TRB FLU, ITRA, VRC, and PSZ against 24 isolates of *C. glabrata* with decreased susceptibility to azoles isolated from the oral cavity of AIDS patients being treated with these agents. The identity of the clinical isolates was confirmed by standard biochemical and microbiological procedures, and the strains were stored in water at room temperature until they were used in the study. *C. parapsilosis* ATCC 22019 was used as the control organism in all experiments.

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TRB (Sandoz Ltd., Basel, Switzerland), FLU (Pfizer Inc., New York, N.Y.), VRC (Pfizer Inc.), ITRA (Janssen Pharmaceutica, Beerse, Belgium), and PSZ (Schering-Plough, Kenilworth, N.J.) were obtained as reagent-grade powders from their respective manufacturers. Stock solutions were prepared in polyethylene glycol (TRB, ITRA, and VRC), dimethyl sulfoxide (PSZ), or water (FLU). Serial twofold dilutions of each antifungal agent were prepared as outlined in document M-27A of the National Committee for Clinical Laboratory Standards (9). Final dilutions were made in RPMI 1640 medium buffered with 0.165 M morpholinepropanesulfonic acid (MOPS) buffer (Angus, Niagara Falls, N.Y.). The final concentrations of the antifungal agents ranged from 0.015 to 8 $\mu\text{g/ml}$ for TRB and the azoles ITRA and PSZ, 0.015 to 4 g/ml for VRC, and 0.125 to 64 $\mu\text{g/ml}$ for FLU. Yeast inocula, prepared spectrophotometrically and further diluted in order to obtain a

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TABLE 1. Mode of interaction between TRB and FLU, ITRA, VRC, and PSZ against clinical isolates of *C. glabrata*^a

Isolate no.	MIC (μg/ml) of the following drug(s):												
	TRB	FLU	TRB/FLU	FIC index	ITRA	TRB/ITRA	FIC index	VRC	TRB/VRC	FIC index	PSZ	TRB/PSZ	FIC index
1	>8	>64	2/32	0.37	4	2/1	0.37	4	4/0.06	0.26	2	0.015/2	1
2	>8	>64	2/16	0.25	0.5	0.015/0.5	1	4	2/0.25	0.18	1	2/0.5	0.62
3	>8	>64	>8/>64	2	>8	4/2	0.37	>4	1/4	0.56	4	2/2	0.62
4	>8	>64	0.25/32	0.26	1	1/0.5	0.56	4	1/0.5	0.18	1	4/0.5	0.75
5	>8	64	1/16	0.31	0.5	2/0.25	0.62	2	1/0.5	0.31	1	2/0.5	0.62
6	>8	64	2/16	0.37	0.5	2/0.25	0.62	2	0.5/0.25	0.15	1	2/0.5	0.62
7	>8	64	0.125/32	0.51	0.5	8/0.25	1	4	2/1	0.37	1	8/0.125	0.62
8	>8	>64	1/4	0.09	0.125	0.5/0.125	1.03	1	0.25/0.25	0.26	0.25	2/0.125	0.62
9	>8	64	0.125/64	1.01	>8	4/4	0.5	4	0.015/4	1	8	2/2	0.37
10	>8	16	0.125/16	1.01	0.5	0.25/0.25	0.51	>4	1/1	0.18	0.5	2/0.25	0.62
11	>8	>64	0.125/64	0.51	>8	8/0.5	0.53	4	4/2	0.75	4	8/0.5	0.62
12	>8	>64	1/64	0.51	1	0.015/1	1	4	2/2	0.62	2	0.015/2	1
13	>8	32	0.5/16	0.56	>8	4/2	0.37	>4	4/1	0.37	4	2/0.5	0.25
14	>8	>64	1/64	0.56	0.5	0.25/0.25	0.51	>4	4/2	0.5	2	0.015/2	1
15	>8	>64	1/64	0.56	>8	8/4	0.75	4	4/2	0.75	8	2/4	0.62
16	>8	16	2/2	0.25	0.5	0.5/0.125	0.28	0.25	2/0.06	0.36	0.5	2/0.125	0.37
17	>8	>64	8/64	1	8	4/4	0.75	>4	4/>4	1.25	4	4/2	0.75
18	>8	32	0.5/16	0.53	0.25	1/0.125	0.56	2	0.06/1	0.5	0.5	4/0.125	0.5
19	>8	>64	0.5/32	0.28	1	0.06/0.5	0.5	>4	2/1	0.25	0.5	8/0.25	1
20	>8	>64	>8/>64	2	>8	1/4	0.31	>4	2/4	0.62	2	4/0.5	0.5
21	>8	64	0.125/54	1.01	<0.125	0.015/<0.125	1	4	2/1	0.37	1	2/0.125	0.25
22	>8	64	2/32	0.62	8	4/1	0.37	4	0.015/4	1	8	2/4	0.62
23	>8	>64	8/64	1	>8	4/2	0.37	>4	8/0.25	0.53	8	2/4	0.62
24	>8	64	8/32	1	4	8/2	1	2	0.015/2	1	4	4/2	0.75

^a TRB, Terbinafine; FLU, fluconazole; ITRA, itraconazole; VRC, voriconazole; PSZ, posaconazole; TRB/FLU, terbinafine in combination with fluconazole; TRB/ITRA, terbinafine in combination with itraconazole; TRB/VRC, terbinafine in combination with voriconazole; TRB/PSZ, terbinafine in combination with posaconazole.

concentration ranging from 1.0×10^3 to 5.0×10^3 CFU/ml (two times the inoculum), were added to each well of the microdilution trays (9). The trays were incubated at 35°C, and the results were read visually at 48 h. MIC endpoints were determined as the first concentration of the antifungal agent tested alone and in combination at which the turbidity in the well was $\geq 90\%$ less than in the control well. Drug interactions were assessed by a checkerboard microdilution method. Drug interactions were classified as synergistic, additive, or antagonistic on the basis of the fractional inhibitory concentration (FIC) index. The FIC index is the sum of the FICs of each of the drugs, which in turn is defined as the MIC of each drug when used in combination divided by the MIC of the drug when used alone. The interaction was defined as synergistic if the FIC was ≤ 0.5 , additive if the FIC index was >0.5 to 2, and antagonistic if the FIC index was >2 (5). Both on-scale and off-scale results were included in the analysis. For computation of the FIC index, high off-scale MICs (>8 μg/ml for TRB, ITRA, and PSZ, >64 μg/ml for fluconazole, and >4 μg/ml for VRC) were converted to the next highest concentration (16, 128, and 8 μg/ml, respectively). When the MIC was off the bottom of the scale, the MIC was assumed to be the lowest MIC tested (1, 2).

The MICs of TRB for the 24 clinical isolates of *C. glabrata* were >8 μg/ml for all the isolates tested. FLU MICs ranged from 16 to >64 μg/ml (MIC at which 50% of the isolates tested were inhibited [MIC₅₀], ≥ 64 μg/ml; MIC₉₀, ≥ 64 μg/ml). ITRA MICs ranged from 0.125 to >8 μg/ml (MIC₅₀, 1 μg/ml; MIC₉₀, 16 μg/ml). VRC MICs ranged from 0.25 to >4 μg/ml (MIC₅₀, 4 μg/ml; MIC₉₀, 8 μg/ml). PSZ MICs ranged from 0.25 to >8 μg/ml (MIC₅₀, 2 μg/ml; MIC₉₀, 8 μg/ml) (Table 1). The calculated MICs of the control were within an acceptable range

for the drugs tested. When TRB was given in combination with FLU, 33.3% (8 of 24) of the interactions were synergistic and 66.6% of the interactions were additive, while antagonism was not observed. TRB MIC was reduced to ≤ 2 μg/ml in 79% of the isolates upon combination with FLU. In the case of FLU, the MIC was reduced to ≤ 32 μg/ml for 50% of the isolates for which initial FLU MICs were ≥ 64 μg/ml upon combination with TRB. When TRB was given in combination with ITRA, 37.5% (9 of 24) of the interactions were synergistic and 62.5% of the interactions were additive, while antagonism was not observed. TRB MIC was reduced to ≤ 2 μg/ml in 58% of the isolates upon combination with ITRA. In case of ITRA, the MIC was reduced to ≤ 0.5 μg/ml in 21% of the isolates for which initial ITRA MICs were ≥ 1 μg/ml when combined with TRB. When TRB was given in combination with VRC, 58% (14 of 24) of the interactions were synergistic and 42% of the interactions were additive, while antagonism was not observed. TRB MIC was reduced to ≤ 2 μg/ml in 71% of the isolates upon combination with VRC. For VRC, the MIC was reduced to ≤ 0.5 μg/ml in 30% of the isolates for which initial VRC MICs were ≥ 1 μg/ml upon combination with TRB. When TRB was given in combination with PSZ, 25% (6 of 24) of the interactions were synergistic and 75% of the interactions were additive, while antagonism was not observed. TRB MIC was reduced to ≤ 2 μg/ml in 62% of the isolates upon combination with PSZ. In the case of PSZ, the MIC was reduced to ≤ 0.5 μg/ml in 47% of the isolates for which initial PSZ MICs were ≥ 1 μg/ml upon combination with TRB.

In our study, we have investigated the in vitro interaction between TRB and four triazoles, FLU, ITRA, VRC, and PSZ, with a large number of *C. glabrata* isolates obtained from HIV patients with OPC undergoing azole therapy. Although pre-

liminary data collected in vivo indicate that oral TRB monotherapy (250 mg/day) for OPC in AIDS patients is not an effective treatment, previous reports have demonstrated that the in vitro combination of TRB with azoles such as FLU, ITRA, or VRC results in a significant synergistic effect against *C. albicans* (1, 14). The theoretical explanation for such effect would be the inhibition of ergosterol biosynthesis at different levels. The azoles inhibit fungal growth by blocking the synthesis of ergosterol, the primary fungal cell sterol, through inhibition of the cytochrome P450-dependent 14- α -demethylase, which is the enzyme responsible for the conversion of lanosterol to ergosterol (13). The mechanism of action of TRB involves the inhibition of the non-cytochrome P450 enzyme squalene epoxidase, resulting in ergosterol deficiency and accumulation of intracellular squalene which disrupts fungal cell membranes (12). To better assess the possible in vitro beneficial effect of TRB on the triazoles, we selected *C. glabrata* strains with reduced susceptibility to azoles. Our in vitro results confirmed a limited activity of TRB when used alone, having a GM MIC of $>8 \mu\text{g/ml}$. These MICs are severalfold higher than the achievable TRB levels in serum (6). On the other hand, our data indicate a clear enhancement of the in vitro activity of the allylamine when combined with the triazoles, decreasing the MICs to levels achievable in serum (58% of the isolates when combined with ITRA, 71% when combined with VRC, and 62% when combined with PSZ). The combination that resulted in a higher number of synergistic interactions was TRB with VRC. The fact that all the interactions of TRB with the four azoles against *C. glabrata* showed synergistic or additive effects, and none showed antagonism, is encouraging. These results support those previously found when combining TRB with FLU and ITRA against isolates of *C. albicans* with reduced susceptibility to azoles (1, 2).

In conclusion, we were able to demonstrate an effective interaction between TRB and triazoles against clinical isolates of *C. glabrata* from the oral mucosa of HIV patients with OPC. Clinical studies are warranted to elucidate the potential utility of this combination therapy in this clinical setting.

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